EXHIBIT G

Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial

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Summary

Background Neovascular, or wet, age-related macular degeneration causes central vision loss and represents a major health problem in elderly people, and is currently treated with frequent intraocular injections of anti-VEGF protein. Gene therapy might enable long-term anti-VEGF therapy from a single treatment. We tested the safety of rAAV.sFLT-1 in treatment of wet age-related macular degeneration with a single subretinal injection.

Methods In this single-centre, phase 1, randomised controlled trial, we enrolled patients with wet age-related macular degeneration at the Lions Eye Institute and the Sir Charles Gairdner Hospital (Nedlands, WA, Australia). Eligible patients had to be aged 65 years or older, have age-related macular degeneration secondary to active subfoveal choroidal neovascularisation, with best corrected visual acuity (BCVA) of 3/60–6/24 and 6/60 or better in the other eye. Patients were randomly assigned (3:1) to receive either 1×10^{10} vector genomes (vg; low-dose rAAV.sFLT-1 group) or 1×10^{11} vg (high-dose rAAV.sFLT-1 group), or no gene-therapy treatment (control group). Randomisation was done by sequential group assignment. All patients and investigators were unmasked. Staff doing the assessments were masked to the study group at study visits. All patients received ranibizumab at baseline and week 4, and rescue treatment during follow-up based on prespecified criteria including BCVA measured on the Early Treatment Diabetic Retinopathy Study (EDTRS) scale, optical coherence tomography, and fluorescein angiography. The primary endpoint was ocular and systemic safety. This trial is registered with ClinicalTrials.gov, number NCT01494805.

Findings From Dec 16, 2011, to April 5, 2012, we enrolled nine patients of whom eight were randomly assigned to receive either intervention (three patients in the low-dose rAAV.sFLT-1 group and three patients in the high-dose rAAV.sFLT-1 group) or no treatment (two patients in the control group). Subretinal injection of rAAV.sFLT-1 was highly reproducible. No drug-related adverse events were noted; procedure-related adverse events (subconjunctival or subretinal haemorrhage and mild cell debris in the anterior vitreous) were generally mild and self-resolving. There was no evidence of chorioretinal atrophy. Clinical laboratory assessments generally remained unchanged from baseline. Four (67%) of six patients in the treatment group required zero rescue injections, and the other two (33%) required only one rescue injection each.

Interpretation rAAV.sFLT-1 was safe and well tolerated. These results support ocular gene therapy as a potential long-term treatment option for wet age-related macular degeneration.

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Introduction

Age-related macular degeneration is the most common cause of visual impairment in high-income countries.[1] The neovascular, or wet, form of the disease is characterised by abnormal choroidal blood vessel growth beneath the macula, which is responsible for high-resolution vision.[2] Wet age-related macular degeneration leads to rapid vision loss and, when left untreated, central blindness.[3] Excessive secretion of VEGF plays a key part in promoting neovascularisation in this disease.[4,5] Additionally, downregulation of anti-angiogenic factors, such as the naturally occurring VEGF-blockers sFLT-1[6] and PEDF,[7] might also promote the disorder, contributing to an imbalance between pro-angiogenic and anti-angiogenic factors potentially underlying the pathophysiology of neovascularisation.[8]

VEGF inhibitors, including pegaptanib sodium,[9] ranibizumab,[10,11] and aflibercept,[12] are safe and effective for slowing disease progression and, in some cases, improving vision. However, such treatments must be administered frequently via intravitreal injection; treatment intervals greater than every 4–8 weeks can result in rapid vision decline.[12–17] When given pro re nata (PRN) or as needed according to a flexible dosing regimen, patients required seven or more injections per year.[14,18] Frequent intravitreal injections are associated with increased cumulative risk of vision-threatening adverse events, including endophthalmitis[19,20] and

intraocular pressure elevation,[21–23] and might increase the risk of arterial thromboembolic events.[24,25] Additionally, the treatment regimen places a substantial burden on patients, physicians, and the health-care system,[26] and patients are systematically undertreated because of difficulties with compliance.[27,28]

Intraocular gene therapy has the potential to enable long-term, stable delivery of therapeutic proteins to treat retinal diseases.[29] Previous studies have shown the use of recombinant adeno-associated vectors (rAAV) to treat Leber's congenital amaurosis[30–32] and choroideraemia,[33] two rare congenital disorders characterised by loss-of-function mutations in intracellular proteins. To treat wet age-related macular degeneration, we developed a strategy to transduce retinal cells with rAAV encoding sFLT-1, a highly potent (dissociation constant KD of about 10 pM), naturally occurring VEGF inhibitor,[34] thus creating a biofactory to deliver therapeutic concentrations of anti-VEGF locally to the macula.

rAAV.sFLT-1 is given via subretinal injection, which places the vector in direct contact with retinal pigment epithelial (RPE) cells and photoreceptors of the outer retina. Although other approaches involve intravitreal administration of rAAV vectors (eg, rAAV2-sFLT01,[35,36] registered with ClinicalTrials.gov, NCT01024998), we chose subretinal injection of rAAV because of its highly efficient expression and limited biodistribution.[37–39] The protein that is produced, sFLT-1, is the soluble form of the VEGFR1 receptor, which binds and inactivates VEGF-A, VEGF-B, and PlGF through its domain 2, the same domain used by the currently marketed therapy aflibercept.[6] We previously showed that rAAV.sFLT-1 enables long-term sFLT-1 secretion[40] and is safe and efficacious in monkey, rat, and mouse models of retinal and choroidal neovascularisation.[41–43] This study reports the first-in-human results with rAAV.sFLT-1 in a clinical trial for wet age-related macular degeneration, and is the first example of rAAV-mediated gene therapy to treat a highly prevalent condition.

Methods

Study design and participants

This single-centre, phase 1, randomised controlled trial investigated the safety and efficacy of a single administration of two different dose concentrations of an rAAV vector of serotype 2 encoding sFLT-1, in patients with wet age-related macular degeneration.

Patients were enrolled under Clinical Protocol 2008-135 version 1.4. Patients were selected from those seen at the Lions Eye Institute and the Sir Charles Gairdner Hospital (Nedlands, WA, Australia). Eligibility criteria included age 65 years or older, age-related macular degeneration secondary to active subfoveal choroidal neovascularisation as evidenced by leakage of fluorescein angiography (FA) and fluid on spectral domain optical coherence tomography (SD-OCT), with best corrected visual acuity (BCVA) of 3/60–6/24 and 6/60 or better in the other eye. Patients with previous anti-VEGF therapy were not excluded, and there was no washout period for anti-VEGF therapy before the baseline visit.

The protocol was approved by the Australian Therapeutic Goods Administration. Appropriate approvals were obtained from the University of Western Australia Institutional Biosafety Committee and Sir Charles Gairdner Hospital Human Ethics Committee. The trial was performed at the Lions Eye Institute in Nedlands, Australia. Throughout the trial the tenets of the Declaration of Helsinki were followed. All patients gave written informed consent.

Randomisation and masking

In step 1, three patients were randomly assigned to low-dose (LD) rAAV.sFLT-1 (1×10^{10} vector genomes [vg]) and one patient to the control group (untreated group; figure 1). After allowing 8 weeks to record any adverse effects from the LD rAAV.sFLT-1 treatment, an additional three patients were randomly assigned to treatment with high-dose (HD) rAAV.sFLT-1 (1×10^{11} vg) and one patient to the control group in step 2. Unequal randomisation of patients to the study groups was implemented primarily to counteract selection bias during study execution, since this phase 1 trial was not powered to detect significant differences in outcome measures between the groups. Randomisation was accomplished by sequential study group assignment according to a randomisation list computer-generated before the study and held off-site. Patients and procedure staff were not masked to treatment received. Staff performing the assessments were masked to the study group of patients at study visits.

A safety evaluation of an 8-week observation period was used between low-dose and high-dose treatments to assess dose dependent safety. This period of time provided an adequate window to allow time for the start of sFLT-1 protein expression and for any potential early immune response to develop.

Procedures

Eligible patients underwent an informed consent process and were enrolled in the study. All patients received an intravitreal injection of 0·5 mg ranibizumab at baseline (day 0) and at week 4 (figure 1). On study day 7 (week 1 visit), patients randomly assigned to intervention received a 100 µL subretinal injection of the appropriate dose of rAAV.sFLT-1 (those randomly assigned to the low dose received 1×10^{10} vg and those assigned to the high dose received 1×10^{11} vg). Controls also had a visit on week 1. Beginning at week 8, subsequent study visits occurred every 4 weeks through to week 52 (total 12 visits). During the follow-up period, patients were permitted retreatment with ranibizumab according to prespecified criteria based on BCVA, SD-OCT, and FA. Laboratory tests included haematology, renal, and hepatic function, electrolytes, urine protein, and IgM, IgG, IgA and lymphocyte subset analysis. Additionally, assays for anti-AAV antibodies,

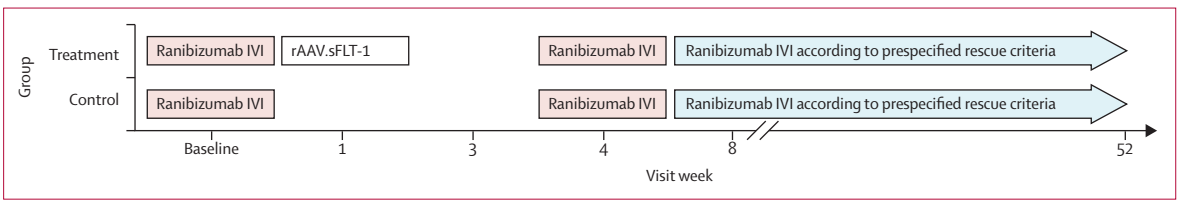

Figure 1: **Study design**
IVI=intravitreal injection. According to prespecified rescue criteria, ranibizumab rescue injection was given if prespecified re-treatment criteria were met, as judged by masked evaluators unaware of the patient's treatment group. Subretinal injection of rAAV.sFLT-1 was done 7 ± 1 days after baseline. Patients were seen postoperatively on days 8, 11–14 (week 2 visit), and 20–24 (week 3 visit). Week 4 and subsequent visits were designated in increments of weeks and occurred at 4-week intervals.

neutralising antibodies, AAV vector shedding, and cellular immunity were also done. Adverse event information was recorded at each visit.

In all patients randomly assigned to receive rAAV.sFLT-1, the subretinal administration procedure was initiated with a standard 23 gauge three-port pars plana approach. A core vitrectomy and induction of posterior vitreous separation from the optic nerve head (if not already present) was done to prevent potential complications. Low-dose or high-dose rAAV.sFLT-1 (appendix) was delivered into the subretinal space via a 41G cannula (23G/0·6 mm, DORC International BV, Zuidaland, Netherlands) in a volume of 100 µL. The injection site was chosen to avoid detachment of the fovea. All patients assigned to gene therapy received topical eye drops of prednisolone and phenylephrine and chloramphenicol four times per day postoperatively for 3 days in the study eye. No other anti-inflammatory or immunosuppressive medications were used.

Rescue criteria were chosen to assess signals of efficacy, protect patient safety, and to assess the long-term treatment effect of rAAV.sFLt-1. Rescue treatment with ranibizumab was given when active choroidal neovascularisation progression was detected, as measured by: (1) loss of ten or more letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) scale from previous visit, or loss of five or more letters from previous visit on ETDRS scale in conjunction with patient perception of functional loss where such loss is attributable to choroidal neovascularisation; (2) any choroidal neovascularisation-related increased subsensory, intraretinal, or sub-RPE fluid on OCT; or (3) signs of increased choroidal neovascularisation leakage on FA.

Outcomes

The primary endpoint was ocular and systemic safety. Ocular safety was monitored at each monthly visit with BCVA, intraocular pressure, slit lamp biomicroscopy, indirect ophthalmoscopy, and SD-OCT, according to the schedule of assessments, with baseline visit deemed as week 0 and the day of surgery as week 1. Signs of visual loss, infection, inflammation, and other safety events including cataract formation and retinal detachment were closely monitored. Patient safety was monitored by periodic physical examinations, vital sign assessment, and routine clinical laboratory testing (complete blood count, comprehensive metabolic panel, lipid panel, and serum electrophoresis measurement). Study data and adverse events were monitored by a data safety monitoring committee with expertise in retinal diseases and gene therapy vectors.

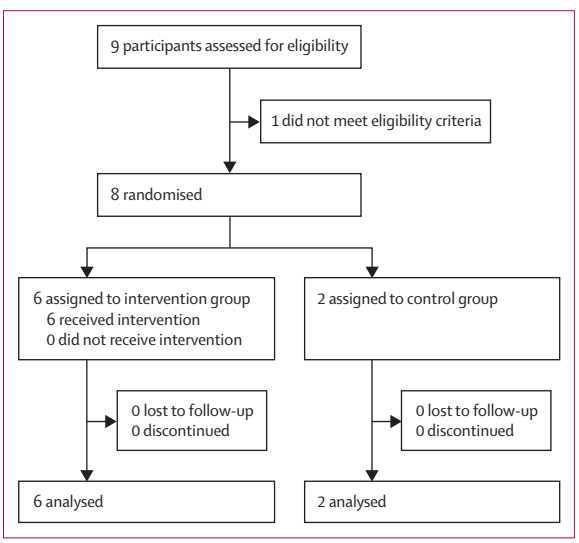

Figure 2: **Trial profile**

Secondary endpoints, assessed on low-dose and high-dose rAAV.sFLT-1 groups combined, included the requirement for rescue therapy, BCVA, and centre point thickness (CPT). SD-OCT was done with the HRA2 (Heidelberg Engineering, Heidelberg, Germany). Images were acquired with raster-scanning of 37 sections of the central portion of the retina and stored for later analysis. The Heidelberg SD-OCT used measures both CPT and surrounding 1 mm subfield thickness automatically. Findings from an earlier study[44] showed a reasonable correlation between CPT and subfield thickness. Since CPT better represents foveal function and BCVA was an important secondary endpoint, we manually confirmed the central foveal slice for remeasurement on every monthly visit.

Statistical analyses

The purpose of this study was to address the safety of the rAAV.sFLT-1 gene therapy treatment and to provide a set of human treatment results to aid in the design of a second larger study. Therefore, we did no sample size calculations. This trial was registered with ClinicalTrials.gov under NCT01494805.

See Online for appendix

	Treatment group	Gender	Ethnic origin	Age (years)	Baseline best corrected visual acuity (ETDRS Letters)	Baseline centre point thickness (μm)	Anti-VEGF injections before study	Time from last pre-study anti-VEGF injection (months)
Patient 1	LD	Female	White	74	33	497	3	60
Patient 2	LD	Female	White	81	28	657	22	1
Patient 3	Control	Male	White	73	28	896	1	2
Patient 4	LD	Male	White	77	46	193	29	2
Patient 5	HD	Male	White	86	56	268	19	1
Patient 6	HD	Female	White	86	54	601	4	1
Patient 7	Control	Female	White	71	39	352	3	1
Patient 8	HD	Male	White	83	34	1094	25	1
Median (IQR)	..	..	..	79 (74–85)	37 (31–50)	549 (310–777)	12 (3–24)	..
Median (IQR) for treatment group only	..	..	..	82 (77–86)	40 (33–54)	549 (268–657)	21 (4–25)	..

ETDRS=Early Treatment Diabetic Retinopathy Study. LD=low dose. HD=high dose.

Table 1: **Baseline demographics and clinical characteristics of patients by patient number**

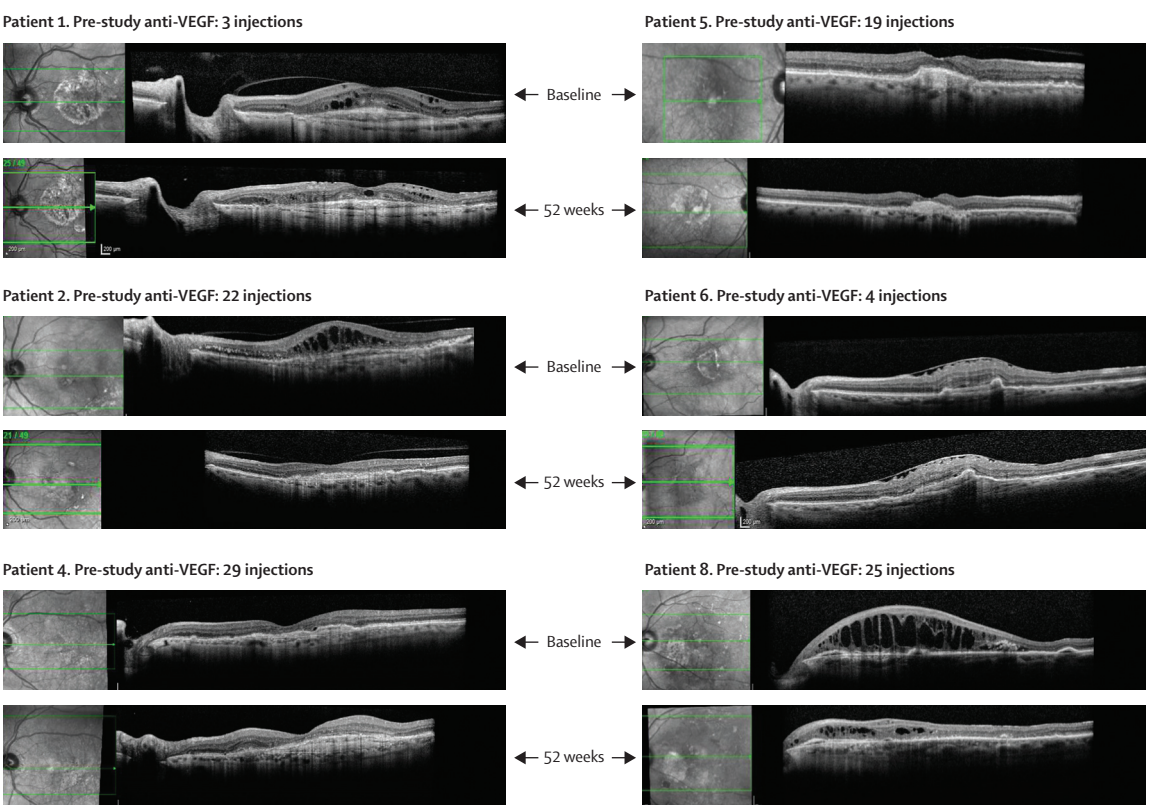

Figure 3: **Comparative optical coherence tomography images of treatment group patients at baseline and at 52 weeks**
Location of each scan on the patient's retina is shown.

Role of the funding source

Avalanche Biotechnologies, Inc had no role in data analysis and interpretation and the corresponding author had full access to all the data in the study. TWC from Avalanche Biotechnologies, Inc made some contributions towards the discussion without interfering with data interpretation. The corresponding author takes responsibility for the decision to publish the results.

Results

From Dec 16, 2011, to April 5, 2012, we enrolled nine patients of whom eight were randomly assigned to

receive either intervention (low-dose rAAV.sFLT-1 [n=3] and high-dose rAAV.sFLT-1 [n=3]) or a control regimen (n=2; figure 2). Patients 1, 2, and 4 were randomly assigned to low-dose rAAV.sFLT-1; patients 5, 6, and 8 to high-dose rAAV.sFLT-1; and patients 3 and 7 to control; appendix). The median age of participants in the treatment group was 82 years (IQR 77–86), and all patients in the treatment group had a confirmed diagnosis of wet age-related macular degeneration with median of 21 previous anti-VEGF injections (IQR 4–25, table 1). Since study eyes had been previously treated, lesion classification into categories such as occult or classic was not done. The median treatment group BCVA at baseline was 40 ETDRS letters (IQR 33–54) and median baseline CPT was 549 µm (IQR 268–657; table 1).

Subretinal injection was successfully done in all six patients treated with rAAV.sFLT-1 and was confirmed by direct visualisation. The investigator (IJC) identified intraoperatively the safest location for the subretinal injection, considering accessibility and other factors. The procedure was well tolerated in all treated patients, and the subretinal bleb was absorbed within 24 h (appendix). No ocular or systemic adverse events attributed to rAAV.sFLT-1 were noted. Multiple assessments up to and including week 52 showed no evidence of ocular inflammation in the anterior segment or posterior segment, and no substantial intraocular pressure elevation or retinal detachment. OCT scans at baseline and at week 52 showed that, for each patient, retinal thickness either visibly improved (patients 1, 2, 6, and 8) or remained stable (patients 4 and 5; figure 3).

No cardiovascular or other systemic adverse events were recorded. Adverse events related to the study procedures were noted in three of six patients, including subconjunctival haemorrhage (patients 1 and 4), subretinal haemorrhage at the injection site (patient 4), and mild cell debris in the anterior vitreous (patient 8); these adverse events were minor and transient, did not require intervention, and did not affect vision. Of the six rAAV.sFLT-1-treated patients, patients 1 and 4 with early stage cataracts had not undergone cataract surgery before enrolment (phakic), and the remaining four had undergone cataract surgery with intraocular lens implantation before baseline (pseudophakic). The two phakic patients developed progression of nuclear cataracts, as expected following vitrectomy in this age group. Cataracts were removed at week 44 (patient 1) and week 36 (patient 4) after injection, with no discernible effect on the macula. Before cataract removal, patient 1 improved from 33 ETDRS letters to 40 letters, then decreased to 29 letters because of cataract progression; after cataract removal, patient 1 returned to 40 letters at week 52 (figure 4). Similarly, patient 4 improved from 46 letters to 57 letters, then decreased to 45 letters because of cataract progression; following cataract removal, patient 4 improved to 58 letters (figure 4). Therefore, cataract removal restored patients to their full visual potential, but was not solely responsible for the vision gain observed during the course of the trial.

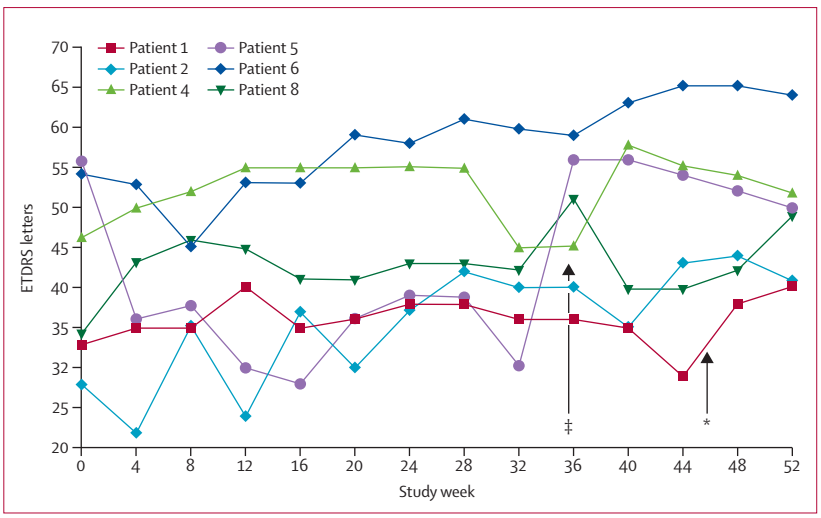

Figure 4: **Display of visual acuity over follow-up period**
ETDRS=early treatment diabetic retinopathy study. Visual acuity was assessed by measuring ETDRS letters at study visits for each patient. The median ETDRS letter at each timepoint for all six treatment group patients. *Patient 1 had a cataract removed from the study eye between the week 44 and the week 48 visits; †Patient 4 had a cataract in the study eye removed 1 day before the week 36 visit.

Several other adverse events, including two serious adverse events (confusion after urinary tract infection in patient 6 and sinus bradycardia in patient 8) were deemed unrelated to gene therapy or study procedures.

We assayed vector shedding, both for vector DNA and viral capsid proteins, in samples of serum, urine, saliva, and tears (from both study and fellow eyes). We assessed the rAAV.sFLT-1 vector DNA copy number by quantitative PCR (qPCR) and detected DNA only in tear specimens obtained 1 day after injection from the injected eye of patients 1 and 5 (appendix). The vector was undetectable by the next timepoint at week 3 and no other qPCR samples were positive. AAV capsid ELISA did not reveal any evidence of capsid proteins (data not shown). Serum concentrations of VEGF, measured by ELISA, remained unchanged over the course of the study. The median circulating VEGF concentration in the treatment group at baseline was 296 pg/mL (range 169–471) and 267 pg/mL (73–541) at week 52.

One patient in the treatment group (patient 2) tested positive at baseline for neutralising antibodies against AAV2 (table 2). Five of six patients in the treatment group had no changes in anti-AAV2 antibody titres. Patient 6 had an increase in anti-AAV2 neutralising antibodies at the week 3 visit; neutralising antibodies remained increased compared with baseline, but were not associated with clinical findings related to safety or efficacy. We measured the frequency of AAV-specific T cells by IFNγ ELISpot assay.[45] Whereas patient 4 showed a transient increase of AAV-specific T-cell concentration at week 4, reactive T cells in the other patients did not exceed the specificity threshold, or were not significantly increased compared with baseline (appendix).

	Screen/Baseline	Day 1	Week 3	Week 4	Week 8	Week 12	Week 16	Week 24	Week 36	Week 52
Patient 1										
Neutralising Ab	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
Total Ab	0·03	0·29	0·36	0·62	0·42	0·59	0·46	0·15	0·14	0·14
Patient 2										
Neutralising Ab	1:20–1:100	1:20–1:100	1:20–1:100	1:20–1:100	1:20–1:100	1:20–1:100	1:20–1:100	1:20–1:100	1:20–1:100	1:20–1:100
Total Ab	0·75	0·52	0·83	0·42	0·64	0·84	0·60	0·56	0·69	0·57
Patient 4										
Neutralising Ab	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
Total Ab	0·22	0·11	0·52	0·17	0·11	0·06	0·07	0·07	0·04	0·05
Patient 5										
Neutralising Ab	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
Total Ab	0·50	0·61	0·17	0·07	ND	0·26	0·30	0·14	0·21	0·55
Patient 6										
Neutralising Ab	<1:20	<1:20	1:20–1:100	1:20–1:100	ND	1:20–1:100	ND	1:20–1:100	1:20–1:100	1:20–1:100
Total Ab	0·79	1·04	1·28	1·55	ND	1·69	ND	0·76	1·07	1·14
Patient 8										
Neutralising Ab	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20	<1:20
Total Ab	0·26	0·40	0·74	0·61	0·45	0·82	0·34	0·83	0·59	0·52

Data for neutralising antibodies (Ab) are presented as titres and data for the total anti-AAV2 antibodies and values are presented as normalised absorbance at 450 nm. ND=not done.

Table 2: **Analysis of anti-AAV antibodies**

	Baseline	Week 52	Change
Patient 1	33	40	+7
Patient 2	28	41	+13
Patient 4	46	52	+6
Patient 5*	56	50	−6
Patient 6	54	64	+10
Patient 8	34	49	+15
Median (IQR)	40 (33 to 54)	49·5 (41 to 52)	+8·5 (+6 to +13)

*Subfoveal fibrovascular scar.

Table 3: **Best corrected visual acuity in ETDRS letters at baseline and at week 52**

Before enrolment, most patients required extensive treatment with anti-VEGF (table 1). During the 52 week follow-up period, each patient was examined monthly for need to be rescued with ranibizumab. Prespecified rescue criteria were based on BCVA, OCT, and FA as assessed by personnel masked to the patient's assignment to treatment group. Of the six patients in the treatment group, four required no ranibizumab injections and the other two required a single injection each (appendix). Thus, the treatment group had two injections in six patients over the course of a year, for an annualised per patient average of 0·33 rescue injection.

The week 52 assessment in the rAAV.sFLT-1-treated patients showed no evidence of vision loss, retinal or pigment epithelial atrophy in the paramacular area of the bleb site, and no evidence of retinal thinning when assessed by OCT. The median CPT was 549 µm (IQR 268–657) at baseline, decreasing to 311 µm (238–450) at week 52 (appendix). FA assessment showed no recurrence of leakage during the year in five of the six treated patients; in one patient, increased leakage at week 52 resulted in a rescue anti-VEGF injection.

BCVA scores improved in five of the six patients treated with rAAV.sFLT-1 compared with the baseline visit, with three of six gaining ten letters or more and one of six gaining 15 letters or more (table 3 and figure 4). Patient 5 had fibrovascular scar tissue that included the subfoveal region at baseline. This patient improved anatomically, recovered BCVA to baseline levels at week 52, remained fluid-free on SD-OCT, and had decreased choroidal neovascularisation leakage on FA. However, retinal health at the fovea might have limited the patient's ability to improve in visual acuity. The BCVA in the treatment group improved from a median of 40 EDTRS letters (range 33–54) at baseline to 50 EDTRS letters (41–52) at week 52.

Discussion

This phase 1 study in six patients treated with rAAV.sFLT-1 supports the safety and tolerability of subretinal rAAV for the treatment of wet age-related macular degeneration. Subretinal injection resulted in a temporary partial detachment of the retina at the site of injection that healed within a day in all patients. The safety data reported here are consistent with previous reports using rAAV for gene therapy in the retina (panel).

As reported in other studies,[30–32] only transient, mild intraocular inflammation following surgery was noted and no dose-limiting toxic effects were encountered. Also

consistent with previous studies,[30–32] the presence of vector was limited to the injected eye 1 day after treatment. Similarly, the immune response noted was limited, with no clear association with any clinical observations. The immunological safety of rAAV vectors noted in this study is reinforced by findings of another report,[31] which showed no immune response following a second administration of rAAV vectors to a contralateral eye, where the potential for significant immune responses based on previous exposure might be enhanced. Our study provides additional reassurance about the positive immunological safety profile of rAAV as a gene therapy vector.

Although an association between monthly anti-VEGF treatment and geographic atrophy has been suggested by retrospective analysis of data from large anti-VEGF trials such as IVAN, CATT, HARBOR,[46–49] there is no evidence that this geographic atrophy is caused by anti-VEGF therapy as opposed to progression of natural age-related macular degeneration or potential artifact,[47,50] and, to our knowledge, no study so far has formally assessed this relation. The best visual outcomes in these studies, and in several other studies of alternative dosing regimens,[12–17] have consistently been achieved with monthly anti-VEGF therapy, and growth of geographic atrophy is not accelerated with monthly therapy compared with PRN therapy[51] or the natural history seen in patients with age-related macular degeneration.[52] Concerns have been raised regarding systemic effects of anti-VEGF drugs on the cardiovascular system following intraocular administration.[24] In this study, however, we did not detect any systemic safety issues or a change in circulating concentrations of VEGF in patients in the treatment group, suggesting that subretinal rAAV.sFLT-1 does not affect VEGF concentrations systemically. The safety data in this phase 1 study support further investigation of rAAV.sFLT-1 as a potential long-term therapy for wet age-related macular degeneration.

We also noted encouraging preliminary signs of improved clinical outcome measures. During the 1-year follow-up period, four (67%) of six treated patients did not require any anti-VEGF rescue injections and two (33%) of six required only one anti-VEGF rescue injection, for an annualised average of 0·33 injections per patient. One of the controls with wet age-related macular degeneration required five rescue injections, the other one had vitreomacular traction and data could not be generated (appendix). Although there are limitations of cross-trial comparisons, it may be instructive to view these data in light of the large body of data from patients on anti-VEGF therapy, including the CATT study.[13,18,19] In these published studies, patients treated with PRN ranibizumab required frequent injections based on VA, OCT, and FA criteria. In the CATT study,[49] for example, the number of rescue injections was similar irrespective of whether patients were treatment naive (6·9 injections), treated PRN during the first year (5·7 injections), or treated during the first year with monthly injections (5·0 injections). In rAAV.sFLT-1-treated patients, CPT, a highly sensitive measure of disease recurrence, improved or was maintained throughout the 1-year follow-up period. CPT was selected as a practical secondary endpoint as it best describes the anatomical status of the area responsible for BCVA, and also an acceptable correlation had been shown between the CPT and the subfield surrounding area in wet age-related macular degeneration.[44]

Finally, BCVA was maintained or improved in all patients treated with rAAV.sFLT-1. In previous studies, such as the VIEW trial examining aflibercept given every 8 weeks,[12] CPT had been shown to increase when anti-VEGF therapy was withheld beyond 4-week intervals. In the CATT study,[49] when patients were switched from monthly to PRN dosing, CPT increased by 31 μm and visual acuity declined by an average of 1·8 letters. Collectively, our data are consistent with previous findings of rAAV.sFLT-1 in animal models of wet age-related macular degeneration, including long-term expression and therapeutic effect,[41,43] as well as data from other human and animal studies investigating subretinal rAAV.[30–32]

***Panel*: Research in context**

Systematic review

We did a systematic review of the evidence published up to July 24, 2014, relevant to gene therapy of wet age-related macular degeneration in PubMed. We searched for clinical studies in which exudative age-related macular degeneration was the patient condition, gene therapy for diseases of the retina, and gene therapy for age-related macular degeneration. Articles considered excluded review articles, editorials, case studies, animal studies, and non-English language articles.

Reviewed wet age-related macular degeneration clinical trials covered various therapies, including surgical, laser ablation, and, more recently, intravitreal injection of anti-VEGF therapies. Reports of gene therapies designed to treat eye disease have used several viral vectors, with rAAV vectors being the most common. We did not find any published study that used an rAAV vector to treat wet age-related macular degeneration with a VEGF antagonist. We identified a single report[53] of a phase 1 clinical trial of gene therapy using an adenoviral vector designed to overexpress PEDF to treat exudative age-related macular degeneration. Treated patients had mild, transient intraocular inflammation, and elevated intraocular pressure. In patients treated with intravitreal injection with less than 1×10^8 particle units of AdPEDF.11, decreases in visual acuity and increases in the size of choroidal neovascularisation lesions were observed. Most patients treated with more than 1×10^8 particle units experienced no change to either their visual acuity or choroidal neovascularisation lesion size.

Interpretation

Anti-VEGF therapy delivered by intravitreal injection is standard of care for wet age-related macular degeneration. No other report of gene therapy using rAAV to deliver anti-VEGF therapy for wet age-related macular degeneration was identified. The results from the first year of this study confirm the good safety profile of rAAV delivered by subretinal injection and suggest efficacy for gene therapy to treat exudative age-related macular degeneration. This study represents an important next step toward gene therapy for chronic diseases of the eye.

Patients enrolled in this study were not treatment naive, and had received several anti-VEGF injections before enrolment. There was no washout period, since the goal of the study was to assess patients who were currently under treatment with standard anti-VEGF drugs. Because of the chronic nature of wet age-related macular degeneration, patients rarely stabilise and often require regular injections even after extensive regular treatment. In the CATT study,[49] for example, patients who were treated with monthly injections for 1 year and then transitioned to PRN therapy still required 5·0 injections in their second year.[49] Thus, a transition from active choroidal neovascularisation following several previous anti-VEGF injections to injection independence without vision decline could provide a powerful means to assess the effectiveness of anti-VEGF gene therapy.

This study was designed as a phase 1 study to assess the safety of the subretinal procedure and rAAV.sFLT-1. Hence, it was not powered to draw definitive conclusions about differences in efficacy between groups. rAAV.sFLT-1 was well tolerated at both doses tested, and there were no meaningful safety differences detected between groups. Results were also similar between treatment groups in terms of CPT, BCVA, and number of rescue treatments. Future dose-ranging studies will provide more information about the relative safety and efficacy between doses. Because of its design as an early stage phase 1 clinical trial, outcomes such as microperimetry and fundus autofluorescence were not recorded, but will be recorded in future trials. The small control group did not receive sham treatment, which limited its application to data analysis. The fact that this study was done in one centre, with the same surgeon doing all surgeries, also affects the generalisability of the results. Future assessments beyond week 52 will be needed so that conclusions regarding the stability of response can be made.

In summary, subretinal delivery of rAAV.sFLT-1 was safe and well tolerated at 1 year in this population of patients with wet age-related macular degeneration. The results of this study support the concept that ocular gene therapy might be a viable long-term treatment option for wet age-related macular degeneration, and add to the growing body of evidence for the viability of intraocular gene therapy to treat retinal disease.

Contributors

EPR participated in the conceptual design. EPR, C-ML, MAD-E, SDS, MSB, TWC, and IJC participated in the study design. EPR, C-ML, CMP, TWC, MAD-E, and IJC contributed to regulatory approval. EPR, C-ML, ALM, CMP, MAF, and IJC contributed to data collection. IJC did the surgeries. EPR, C-ML, ALM, MEW, MAD-E, MAF, and IJC participated in data analysis. EPR, C-ML, ALM, MEW, MAD-E, TWC, and IJC participated in the writing of the report.

Declaration of interests

EPR received funding from Avalanche Biotechnologies, Inc during the conduct of the study; additionally, she has a patent pending and acts as the Chair of the Scientific Advisory Board for Avalanche Biotechnologies, Inc. C-ML has a patent pending, reports grants from National Health and Medical Research Council of Australia, from Avalanche Biotechnologies Inc, and from Richard Pearce Bequest, during the conduct of the study. ALM reports personal fees and non-financial support from Avalanche Biotechnologies, Inc during the conduct of the study. SDS reports other from Avalanche Biotechnologies, Inc during the conduct of the study. MSB reports equity holder, member of the Board of Directors and compensation for the Board for Avalanche Biotechnologies, Inc, equity holder for Ovuleve Corporation and Digisight Corporation, outside the submitted work; additionally he has a patent on ocular gene therapy using Avalanche-related transfection issued. TWC reports personal fees from Avalanche Biotechnologies, Inc, outside the submitted work; additionally, he has a patent on treatment of age-related macular degeneration using AAV.sFLT-1 licensed to Avalanche Biotechnologies, Inc. IJC reports funding from Avalanche Biotechnologies, Inc during the conduct of the study; additionally, he has a patent pending and chairs the Avalanche Medical Advisory Board. The other authors declare no competing interests.

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